



Stress correlated factors with water quality under different rearing conditions of the European sea bass (*Dicentrarchus labrax*) juveniles

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ABSTRACT

Water exchange rates were manipulated to reduce the negative effect of high stocking density adversely affects the rearing condition such as water exchange rates has to be manipulated to elevate stress factors. European sea bass juveniles with an average body weight of 4.5 g fish/m³ were stocked at three densities (50, 100 and 150 fish/m³) in 12 concrete ponds. Two water exchange rates (20% and 30% of total water volume) were applied for 18 weeks to investigate their influence on reducing the negative effect of stocking density and enhancing the water quality. The results indicated that the 30% water exchange rate was better for achieving high growth performance and feed utilization, regardless of stocking density. Water quality (total ammonia nitrogen, un-ionized ammonia, nitrite and nitrate) was enhanced, dissolved oxygen increased and all harmful nitrogen derivatives decreased when the 30% water exchange rate was applied. Additionally, the 30% water exchange rate significantly increased the water temperature (16.33°C) compared to the 20% exchange rate (14.33°C), and the final body weight, weight gain and specific growth rate were significantly increased ($P<0.05$) with the 30% water exchange rate. The survival rate was 97%, which was significantly the highest at the density of 50 fingerlings/m³ at a water exchange rate of 30%. Although the feed conversion ratio, protein efficiency ratio, and protein productive value significantly improved with the 30% water exchange rate at all densities, the 100 fish/m³ stocking density achieved the best feed conversion ratio. However, the haematological parameters showed a significant increase ($P<0.05$) in haemoglobin, haematocrit, white blood cells, lymphocytes, monocytes and neutrophils with a low stocking density and a 30% water exchange rate

INTRODUCTION

European sea bass are considered the most important economic aquaculture marine species in Europe and in Egypt (Chavanne *et al.*, 2016; Tacon, 2018; Lucas *et al.*, 2019). Several culture systems have been used to produce the highest yield of European sea bass.

One of the most commonly used tools to improve the yield of certain aquaculture areas and reduce the unit cost is increasing the stocking density (SD). Wide ranges of densities are regularly used for cultured sea bass, e.g., from less than 10 to more than 100 kg/m³, based on the rearing conditions and growth phase (Ellis *et al.*, 2002; Conte, 2004). However, water quality is highly influenced by fish density in the culture system (especially in well-water conditions); for example, high densities can cause water quality deterioration and physical and biological stress in fish, especially when they are chronically exposed to high SDs (Björnsson and Ólafsdóttir, 2006; de Oliveira *et al.*, 2012). The SD and water exchange rate (W_{ex}) have a close link with physiological responses in fish (Montero *et al.*, 1999). Thus, a higher W_{ex} can reduce the harmful effects of high stocking densities by enhancing physicochemical parameters such as water temperature and ammonia content (Person-Le Ruyet and Le Bayon, 2009; López *et al.*, 2015; Lanari *et al.*, 2016).

In addition, several studies have suggested that high stocking densities negatively affect growth performance (Di Marco *et al.*, 2008; Costa *et al.*, 2017) and haematological parameters (Coz-Rakovac *et al.*, 2005). Haematological parameters are an important tool used to monitor the health conditions and physiology of fish in culture systems (Ballarin *et al.*, 2004; Tavares-Dias *et al.*, 2007; Fazio *et al.*, 2015a; Fazio *et al.*, 2015b) under chronic stress (Bahmani *et al.*, 2001; Cnaani *et al.*, 2004; Zarejabad *et al.*, 2010).

Recently, the decreasing water sources available to aquaculture have gradually resulted in a greater interest in well water and purified water (Ercan *et al.*, 2015).

Therefore, this paper aimed to investigate the effect of chronic stress, resulting from different rearing conditions, on European sea bass performance; additionally, the water quality of cultured concrete ponds was monitored.

MATERIALS AND METHODS

1. Experimental fish:

Fish were obtained from a government hatchery located west of Alexandria. After two weeks of acclimation, sea bass fingerlings (average weight: 4.5 gm) were stocked in 0.5 m³ hapas, which were fixed in concrete ponds (2×3×0.5 m). The experimental period was 14 weeks. Fish were weighed biweekly to adjust the amount of feed and to determine the biological status of the fish. Additionally, experimental fish were fed to satiation on a commercial diet containing 42% crude protein.

2. Experimental conditions:

The experiment was carried out in El Max Station for Applied Research, NIOF (National Institute of Oceanography and fisheries), Alexandria branch, Egypt. Saline well water was used in the experiment and obtained from an 80 m deep saline well with 28‰ salinity.

This experiment was designed as two-way ANOVA when two factors were investigated: SD and W_{ex} . Eighteen concrete ponds were used for seabass fingerling stocking. Treatments were distributed as follows: 20% and 150 fingerlings/m³ (20% W_{ex} , high density (HD)), 20% and 100 fingerlings/m³ (20% W_{ex} , moderate density (MD)), 20% and 50 fingerlings/m³ (20% W_{ex} , low density (LD)), 30% and 150 fingerlings/m³ (30% W_{ex} , HD), 30% and 100 fingerlings/m³ (30% W_{ex} , MD) and 30%

and 50 fingerlings/m³ (30% W_{ex} , LD). Also, each treatment represented in three replicates.

Table (1). Chemical analysis of the experimental diet.

Chemical composition% on dry matter basis	
Dry matter (DM)	88
Crude protein (CP)	42
Ether extract (EE)	21
Crude fibre (CF)	2
Ash	7
NEF*	16
*NFE = 100 – (% Moisture + % Ash + % Lipid + % Protein)	

*NFE: Nitrogen free extract (NRC, 1993).

3. Growth performance parameters:

Fingerlings from each treatment were weighed biweekly to verify the weight gain and adjust the amount of provided feed.

The growth performance and feed utilization parameters were calculated according to the following equations:

Weight gain (g/fish): $WG = W_t - W_0$

where W_0 is the initial mean weight of fish, in grams, and W_t is the final mean weight of fish, in grams.

Average daily gain (g/fish/day): $ADG = (W_t - W_0)/n$

where n is the duration period.

Specific growth rate (%/day): $SGR = 100 \times (\ln W_t - \ln W_0)/\text{days}$

where ln is the natural logarithm.

Survival rate (%) = $100 \times (\text{initial number of fish}/\text{final number of fish})$.

Feed intake (FI) (g/fish): This is the amount of feed given or supplied during the experimental period and was determined on a gram per fish basis.

Feed conversion ratio (FCR) = dry matter intake (g)/weight gain (g).

Protein efficiency ratio (PER) = total weight gain (g)/amount of protein fed (g).

4. Proximate analysis:

Homogenized samples were analysed in triplicate for moisture, protein, lipids and ash. Protein analysis was conducted according to (AOA, 2000), while moisture (fish sample) was determined by oven drying at 110°C to constant weight. Ash content was evaluated by combustion in a muffle furnace at 550°C.

5. Water parameters:

The temperature and pH were measured using a portable pH meter (pH-8424-HANNA Instrument). Dissolved oxygen (DO) was measured with an HI-9142 (HANNA Instrument). The concentration of total ammonia nitrogen (TAN) was analysed using YSI 9300 photometer and YSI Professional Plus.

6. Blood parameters:

Sea bass fingerlings were anaesthetized with clove oil (0.3 mg/L) to prepare fish specimen for blood collection. The blood samples were collected from the caudal vein

using syringes and then transferred to 1.5 ml tubes. Immediately, blood samples were transported to the laboratory to evaluate the red blood cells (RBCs), haemoglobin (Hb), haematocrit (Hct), white blood cells (WBCs), lymphocytes, monocytes and neutrocytes.

7. Statistical analysis:

Raw data were transferred to an Excel sheet and then analysed by two-way ANOVA using SPSS 24.00 (SPSS, IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). Significant differences between interactions were analysed by R. The means of data were compared using Tuckey test, ($P \leq 0.05$). Additionally, the data are represented as the mean \pm standard error.

RESULTS:

1. Growth performance:

The growth performance parameter data are shown in Table 2. The final body weight (FBW), WG, ADG, SGR and yield were significantly ($P \leq 0.05$) affected by the W_{ex} (fig.1). All mentioned values were higher in the 30% W_{ex} treatment (23.68 g, 19.23 g, 0.16 g/day, 1.34%/day and 2.17 kg/m³, respectively) than in the 20% W_{ex} treatment (20.75 g, 16.28 g, 0.14g/day, 1.27%/day and 1.52 kg/m³, respectively). In addition, the survival rate was significantly better in the 30% W_{ex} treatment (95.43%) than in the 20% W_{ex} treatment (80.50%).

In the same context, different stocking densities significantly influenced ($P \leq 0.05$) sea bass performance values. The LD treatment group (50 fingerlings/m³) significantly ($P \leq 0.05$) had the best values (25.5 g, 21.01g, 0.18 g/day, and 1.50%/day, respectively) among all three examined stocking densities. The same results were obtained for survival rate, which was the highest (93.75%) in the LD group followed by that in the MD group (87.50%). Nevertheless, sea bass fingerlings grown in the HD group achieved the best yield, with a value of 2.37 kg/m³, which was significantly different ($P \leq 0.05$) from the values obtained in the other stocking densities (1.97 and 1.20 kg/m³, respectively).

In relation to the interaction between SD and W_{ex} , the growth performance values significantly ($P \leq 0.05$) differed between all inspected treatments. The best growth performance parameters (FBW, WG, ADG and SGR) were attained by fingerlings grown at the 50 fingerlings/m³ SD when the daily W_{ex} was 30% of the total water volume (25.8 g, 21.3 g, 0.18 g/day and 1.46%/day, respectively), followed by fingerlings grown at the same SD but with a W_{ex} of 20% (25.2 g, 20.7 g, 0.17 g/day and 1.44%/day, respectively); there were no significant differences ($P \leq 0.05$) between these two groups (20% and 30% W_{ex} at LD). The 30% W_{ex} and 100 fingerlings/m³ density produced the third best growth performance values (24.8 g, 20.4 g, 0.17 g/day and 1.43%/day, respectively), with no significant differences ($P \leq 0.05$) between the best two values. Nevertheless, fingerlings grown in the HD group with 20% W_{ex} significantly ($P \leq 0.05$) had the worst growth parameter values (17.4 g, 13 g, 0.11 g/day and 1.14%/day, respectively).

The survival rate was significantly ($P \leq 0.05$) higher in the 30% W_{ex} under all densities; specifically, the survival rate was highest in the LD group (97%) followed by that in the MD group (95.5%). Nonetheless, the HD and 20% W_{ex} treatment produced the lowest (71.5%) survival rate, as it decreased by 26% relative to the highest value.

The 30% W_{ex} and HD (150 fingerlings/m³) treatment significantly ($P \leq 0.05$) achieved the best yield (2.87 kg/m³), and it represented a 35% increase from the value achieved by the 20% W_{ex} at the same SD (1.86 kg/m³). Additionally, it was obvious that the lowest yields were attained in the LD groups with the different W_{ex} treatments.

2. Feed utilization:

The data on feed utilization are shown in Table 3. Similar to the growth performance parameter data, the FCR, FI, PER and productive protein value (PPV) were significantly ($P \leq 0.05$) affected by the two W_{ex} treatments (fig.2). Their values were better in the 30% W_{ex} treatment (1.25, 23.95 g/fish, 1.92 g and 29.15%, respectively) than in the 20% W_{ex} treatment (1.35, 21.61 g/fish, 1.78 g and 29.15%, respectively). Additionally, the different stocking densities significantly influenced ($P \leq 0.05$) the FCR, FI, PER and PPV. Specifically, the LD group significantly ($P \leq 0.05$) achieved the best FCR (1.25) followed by the MD (1.29), with no significant differences ($P \leq 0.05$) between the two treatments. In addition, the FI was highest in the LD group (26.22 g/fish).

The same results were obtained for the PER and PPV values, i.e., the LD group significantly ($P \leq 0.05$) attained the best values (1.91 g and 34.11%, respectively), and the PPV was approximately 60% higher than the PPV in the highest tested SD (20.29%).

Regarding the interaction between SD and W_{ex} , feed utilization significantly differed ($P \leq 0.05$) among all tested treatments. The best FCR was obtained by European sea bass fingerlings grown in the MD group with the 30% W_{ex} (1.21); this group was followed by the fingerlings grown in the LD group with the 20% W_{ex} (1.22) and the fingerlings grown in the HD group with the 30% W_{ex} (1.25), with no significant differences ($P \leq 0.05$) among these groups. Nevertheless, the worst FCR was obtained in fingerlings grown in the HD group with the 20% W_{ex} (1.45), where the value significantly ($P \leq 0.05$) increased by 20% relative to the best value.

However, the FI did not differ significantly ($P \leq 0.05$) according to the interaction between the two examined factors, and the highest FI was recorded in fingerlings reared in the LD group with the 30% W_{ex} (27.2 g/fish). Furthermore, the best PER was obtained by fingerlings reared in the MD group with the 30% W_{ex} (1.97 g). This value did not differ significantly ($P \leq 0.05$) from that of the 20% W_{ex} under the lowest density (1.95 g) and of the 30% W_{ex} under the highest density (1.91 g). The fingerlings grown in the HD group with the 20% W_{ex} significantly ($P \leq 0.05$) had the lowest PER and PPV (1.64 g and 19%, respectively). Nevertheless, fingerlings reared in the LD group with the 30% W_{ex} significantly ($P \leq 0.05$) achieved the highest PPV (38.3%) among all tested treatments.

Table (2). The effect of different stocking densities and two water exchange rates (W_{ex} %) on growth performance of European sea bass fingerlings

Variable	30% (water exchange rate)			20% water exchange rate			W_{ex} L	<i>P</i> -value group	$W \times G^1$
	High density	Mid density	Low density	High density	Mid density	Low density			
IBW (g)	4.42 ± 0.02	4.48 ± 0.02	4.46 ± 0.06	4.43 ± 0.02	4.5 ± 0.02	4.5 ± 0.06	0.484	0.265	0.924
FBW (g)	20.4 ± 0.78^b	24.8 ± 0.28^a	25.8 ± 0.34^a	17.4 ± 0.61^b	19.7 ± 0.56^b	25.2 ± 0.78^a	0.001	<0.001	0.022
Gain (g)	16 ± 0.76^b	20.4 ± 0.26^a	21.3 ± 0.40^a	13 ± 0.59^b	15.2 ± 0.53^b	20.7 ± 0.84^a	0.001	<0.001	0.024
ADG (g/day)	0.134 ± 0.01^b	0.17 ± 0.00^a	0.18 ± 0.00^a	0.108 ± 0.00^b	0.126 ± 0.00^b	0.173 ± 0.01^a	0.001	<0.001	0.024
SGR (%/day)	1.28 ± 0.03^b	1.43 ± 0.006^a	1.46 ± 0.02^a	1.14 ± 0.03^b	1.23 ± 0.02^b	1.44 ± 0.04^a	0.001	<0.001	0.035
SUR%	93.8 ± 0.80^{ab}	95.5 ± 0.50^a	97 ± 1^a	71.5 ± 0.5^d	79.5 ± 0.50^c	90.5 ± 0.50^b	<0.001	<0.001	<0.001
Y ($kg\ m^{-1}$)	2.87 ± 0.09^a	2.37 ± 0.04^b	1.25 ± 0.003^e	1.86 ± 0.05^c	1.56 ± 0.03^d	1.14 ± 0.0288^e	<0.001	<0.001	<0.001

Values are means \pm SEM, n = 3 per treatment group.

Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

$1W \times G = W_{ex} L \times$ group interaction effect.

Table (3). The effect of different stocking densities and two water exchange rates on feed utilization of European sea bass fingerlings

Variable	30% water exchange rate			20% water exchange rate			W_{ex} L	<i>P</i> -value group	$W \times G^1$
	High density	Mid density	Low density	High density	Mid density	Low density			
FCR	1.25 ± 0.05^b	1.21 ± 0.01^b	1.27 ± 0.03^{ab}	1.45 ± 0.05^a	1.37 ± 0.04^{ab}	1.22 ± 0^b	0.012	0.068	0.022
FI (g/fish)	20.1 ± 1.75^{bc}	24.6 ± 0.11^{ab}	27.2 ± 0.03^a	18.7 ± 0.21^c	20.8 ± 1.34^{bc}	25.3 ± 1.02^{ab}	0.028	0.001	0.466
PER (g)	1.91 ± 0.08^{ab}	1.97 ± 0.02^a	1.87 ± 0.04^{ab}	1.64 ± 0.06^b	1.74 ± 0.05^{ab}	$1.95 \pm 1.57e-16^a$	0.012	0.075	0.019
PPV%	21.6 ± 0.14^{cd}	27.5 ± 1.73^{bc}	38.3 ± 2.13^a	19 ± 1.50^d	22.9 ± 0.28^{bd}	29.9 ± 1.04^b	0.003	<0.001	0.176

Values are means \pm SEM, n = 3 per treatment group.

Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

$1W \times G = W_{ex} L \times$ group interaction effect.

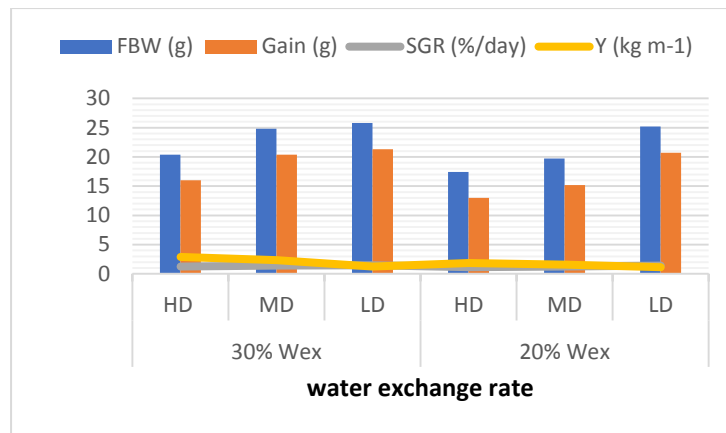


Fig. (1) The effect of SD and W_{ex} on growth performance parameters of European sea bass fingerlings.

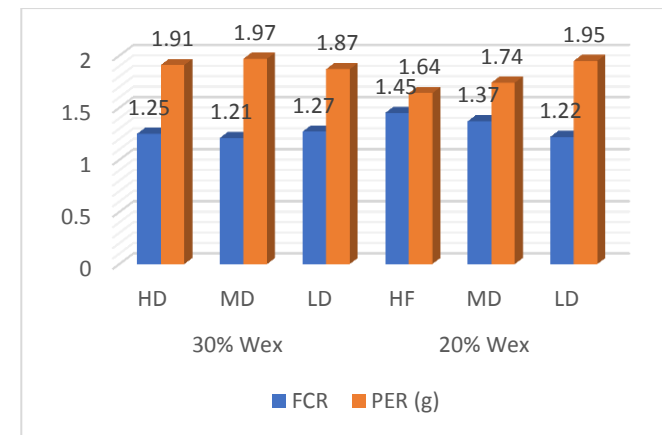


Fig. (2) The effect of SD and W_{ex} on FCE and PER of European sea bass fingerlings.

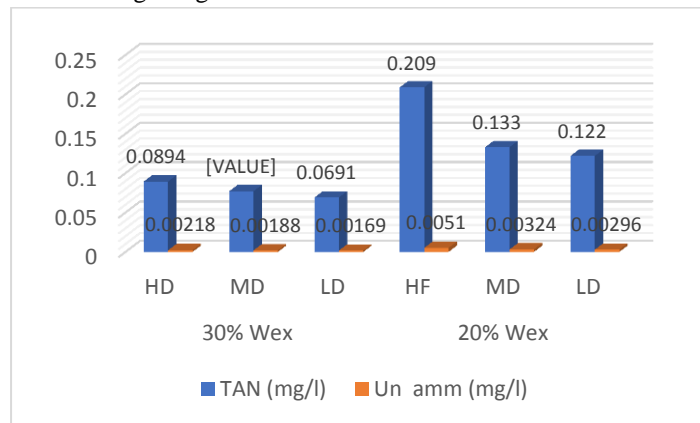


Fig. (3) The effect of SD and W_{ex} on TAN and Un-ionized ammonia of water.

3. Fish body chemical analysis:

Data on the chemical composition of European sea bass fingerlings are presented in Table 4. The data revealed that moisture content did not differ significantly ($P \leq 0.05$) between different W_{ex} treatments and SD treatments.

The dry-matter protein, lipid and ash values differed significantly ($P \leq 0.05$) between the two W_{ex} treatments. A better body content of protein (57.35% of dry matter) was obtained when the W_{ex} was 30% than when the W_{ex} was 20% (49.04% of dry matter). However, the lipid and ash contents were higher in the 20% W_{ex} (25.26% and 15.60% of dry matter, respectively) than in the 30% W_{ex} (20.63% and 11.92% of dry matter, respectively), and these differences were significant ($P \leq 0.05$).

Significant differences were found among the tested SD treatments. The highest protein content was obtained by fingerlings grown in the LD group (57.17% of dry matter), followed by significantly lower values obtained by fish grown in the MD and HD groups (52.99% and 49.42% of dry matter, respectively). However, the highest lipid content was obtained by fingerlings grown in the HD group followed by those grown in the MD group, and these values were significant ($P \leq 0.05$). In addition, significant differences ($P \leq 0.05$) were found for ash content, with the lowest value obtained by fish in the LD group, (24.53%, 23.23% and 21.09% of dry matter for the HD, MD, and LD groups, respectively).

The interaction between the examined factors (SD and W_{ex}) showed significant differences ($P \leq 0.05$) between all examined treatments. Fingerlings grown in the lowest SD significantly ($P \leq 0.05$) achieved the best protein value when the W_{ex} was 30% (61.6% of dry matter), and they were followed by fingerlings grown in the MD group under the same W_{ex} (57% of dry matter). In contrast, the lowest protein value was recorded in fingerlings grown in the HD group with the 20% W_{ex} (45.4% of dry matter).

The highest lipid and ash values were obtained by fish in the HD group with the 20% W_{ex} (27.4% and 17.2% of dry matter, respectively), followed by fish in the MD group (25.2% and 15.7% of dry matter, respectively); the two groups were not significantly different ($P \leq 0.05$). Fingerlings grown in the MD (21.3% and 11.6% of dry matter) and LD (18.9% and 9.63% of dry matter, respectively) groups significantly achieved the lowest lipids and ash contents when the W_{ex} was 30%.

4. Water physicochemical parameters:

The water quality data are shown in Table 5. Both SD and W_{ex} significantly affected ($P \leq 0.05$) water quality (fig.3). The water temperature was significantly higher (16.33°C) when the W_{ex} was 30%; in contrast, the water temperature was 14.33°C when the W_{ex} was 20%. In addition, the W_{ex} significantly affected the TAN, un-ionized ammonia, NO_2 and NO_3 . The 30% W_{ex} produced the best water quality (0.079 mg/l, 0.002 mg/l, 0.24 mg/l and 0.02 mg/l, respectively) and these values were significantly lower than the values produced in the 20% W_{ex} treatment (0.154 mg/l, 0.004 mg/l, 0.38 mg/l and 0.035 mg/l, respectively).

Moreover, different SDs significantly influenced water quality parameters. The highest water temperature was recorded in ponds with the highest SD (16°C), while

the lowest values were recorded in ponds with the middle and lowest SDs (15°C). Furthermore, the TAN (0.149 and 0.105 mg/l, respectively), un-ionized ammonia (0.004 and 0.003 mg/l, respectively), NO₂ (0.394 and 0.282 mg/l, respectively) and NO₃ (0.038 and 0.26 mg/l, respectively) values were significantly higher in both the highest and middle SD groups than in the lowest SD group (0.095, 0.002, 0.242 and 0.022 mg/l, respectively).

In relation to the interaction between the two factors tested, the TAN, un-ionized ammonia, NO₂ and NO₃ values were significantly affected. The highest water temperature was recorded in the 30% W_{ex} + HD treatment, followed by that in the 30% W_{ex} + MD treatment (16°C). Nevertheless, the lowest water temperature was recorded in ponds with the 20% W_{ex} and the LD and MD treatments (14°C), without any significant differences. The TAN, un-ionized ammonia and NO₂ values were significantly lower in the 30% W_{ex} and LD group (0.069, 0.0017 and 0.21 mg/l, respectively), significantly followed by those in the MD group with the same W_{ex} (0.077, 0.0019, 0.22 mg/l, respectively). However, the highest values were obtained with the 20% W_{ex} under HD (0.209, 0.005, 0.51 mg/l, respectively). The lowest NO₃ values were obtained with the 30% W_{ex} under MD and LD (0.021 and 0.022 mg/l, respectively), without any significant differences between the values for the two SDs. Nevertheless, the NO₃ value was significantly highest when the W_{ex} was 20% in the HD group (0.052 mg/l).

5. Plasma and serum parameters:

The serum and plasma parameters are shown in Table 6. The W_{ex} significantly ($P \leq 0.05$) affected the blood parameters, and the RBC, Hb, Hct, WBC lymphocyte, monocyte and neutrocyte values were significantly higher when the W_{ex} was 30% (1.65, 8.28, 22.74, 27.45, 67.24, 2.38 and 20.25, respectively) than when the W_{ex} was 20% (1.19, 6.13, 15.93, 20.73, 46.20, 2.05 and 15.87, respectively). Different SDs significantly influenced ($P \leq 0.05$) the RBC, Hb, Hct, WBC, lymphocyte, monocyte and neutrocyte values. Their values were significantly highest in the LD group (1.56, 7.69, 20.33, 25.63, 8.80, 2.32 and 18.69, respectively) compared with those in the MD group (1.45, .24, 19.72, 23.32, 56.40, 2.25 and 18.11, respectively) and those in the HD group (1.26, 6.69, 17.95, 23.33, 54.96, 2.07 and 17.39, respectively), which had the lowest values ($P \leq 0.05$). Nevertheless, there were no significant differences ($P \leq 0.05$) between the Hct values of both the LD and the MD groups. Additionally, there were no significant differences ($P \leq 0.05$) in the WBC values between the MD and HD groups. Moreover, the interaction between both W_{ex} and SD had significant differences in the plasma and serum parameters. The highest RBC value was recorded in fingerlings grown in the LD group with the 30% W_{ex} (1.84) and was significantly different ($P \leq 0.05$) from that of fish grown in the MD group with the same 30% W_{ex} (1.67). The lowest RBCs value was recorded in fish grown in the HD group with the W_{ex} of 20% (1.06). The same trend was found for the Hb, Hct, WBCs, lymphocytes, monocytes and neutrocyte values. The highest values (9.0, 24.4, 30, 0.8, 2.54 and 1.3, respectively) were recorded in fingerlings grown in the LD group with the 30% W_{ex}, followed by those in fingerlings grown in the MD group with the 30% W_{ex} (8.36, 23.4, 26.2, 66.8, 2.38 and 20.3, respectively); the differences were significant ($P \leq 0.05$) except in the case of Hct. The lowest values (5.9, 15.5, 20.5, 45.8, 1.92 and 15.6, respectively) were recorded in fingerlings grown in the LD group with the 20% W_{ex}. There were no significant differences in monocyte values ($P \leq 0.05$) between the different treatments in relation to the interaction between SD and W_{ex}.

Table (4). The effect of different stocking densities and two water exchange rates on body chemical composition European sea bass fingerlings.

Variable %	30% water exchange rate			20% water exchange rate			W ex L	P-value group	W×G ¹
	High density	Mid density	Low density	High density	Mid density	Low density			
moisture	70.2 ± 0.08	70.3 ± 0.34	69.8 ± 0.59	70.5 ± 1	70.4 ± 0.42	69.7 ± 0.60	0.868	0.544	0.953
Protein	53.5 ± 0.05 ^c	57 ± 0.16 ^b	61.6 ± 0.33 ^a	45.4 ± 0.88 ^e	49 ± 1.12 ^d	52.7 ± 0.35 ^c	<0.001	<0.001	0.717
Lipid	21.7 ± 0.36 ^c	21.3 ± 0.25 ^{cd}	18.9 ± 0.14 ^d	27.4 ± 0.71 ^a	25.2 ± 0.81 ^{ab}	23.3 ± 0.22 ^{bc}	<0.001	0.001	0.228
ash	14.8 ± 0.31 ^b	11.6 ± 0.41 ^c	9.36 ± 0.47 ^d	17.2 ± 0.18 ^a	15.7 ± 0.31 ^{ab}	13.9 ± 0.13 ^b	<0.001	<0.001	0.034

Values are means ± SEM, n = 3 per treatment group.

^{a-e}Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

¹W × G = W ex L × group interaction effect.

Table (5). The effect of different stocking densities and two water exchange rates on water quality used in European sea bass fingerlings rearing ponds.

Variable	30% water exchange rate			20% water exchange rate			W ex L	P-value group	W×G ¹
	High density	Mid density	Low density	High density	Mid density	Low density			
Temperature (c°)	17±5.00 ^a	16±5.66 ^b	16±4.58 ^b	15±4.89 ^c	14±5.21 ^d	14±5.44 ^d	0.001<	0.002	1.000
pH	7.74 ± 0.05 ^{cd}	7.83± 0.10 ^{ab}	8.11 ± 0.10 ^a	7.63 ± 0.05 ^d	7.62 ± 0.05 ^d	7.74 ± 0.05 ^{cd}	0.177	0.429	0.783
TAN (mg/l)	0.089± 0.00 ^d	0.077±0.00 ^e	0.069±0.00 ^f	0.209 ± 0.00 ^a	0.133 ± 0.00 ^b	0.122 ± 0.00 ^c	<0.001	<0.001	<0.001
UnA (mg/l)	0.002±3.8e-05 ^d	0.0019±5.49e-06 ^e	0.0017±8.08e-06 ^f	0.005± 2.29e-05 ^a	0.003 ± 1.4e-05 ^b	0.003± 9.15e-06 ^c	<0.001	<0.001	<0.001
NO ₂ (mg/l)	0.281±0.03 ^{bc}	0.222 ± 0.01 ^{bc}	0.21 ± 0.011 ^c	0.508 ± 0.01 ^a	0.341 ± 0.01 ^b	0.273 ± 0.04 ^{bc}	<0.001	0.001	0.024
NO ₃ (mg/l)	0.024±0.00 ^c	0.021 ± 0.00 ^c	0.0215 ± 5e-04 ^c	0.0515 ± 5e-04 ^a	0.031 ± 0.00 ^b	0.0215 ± 5e-04 ^c	<0.001	<0.001	<0.001

Values are means ± SEM, n = 3 per treatment group.

^{a-e}Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

¹W × G = W ex L × group interaction effect.

DISCUSSION:

Several factors affect fish cultures in concrete ponds. The environmental conditions and genetics have serious effects on fish performance. The SD and W_{ex} are considered the most important environmental conditions that directly affect fish performance.

The present results showed that water quality had a significant and vital influence on the biological and physiological performance of European sea bass. Better water quality led to better performance. High water quality was obtained with a W_{ex} of 30% of the total water volume of the pond, and this W_{ex} significantly decreased the negative effect of increased SD. The TAN, un-ionized ammonia, nitrite and nitrate levels and physical parameters of water were better in the 30% W_{ex} treatment. This result indicates positive improvements in water quality and, in turn, on juvenile European sea bass performance.

The current results show that the 30% W_{ex} reduced all values of harmful nitrogen compounds. The effect of chronic stress on cultured fish was also reduced. However, there were no significant differences in pH, the water temperature values increased significantly in the 30% W_{ex} treatment compared with the 20% W_{ex} treatment at the same SDs. Despite the LD group having the lowest values, the HD group with the 30% W_{ex} achieved better values than the 20% W_{ex} treatment at the same density. The 30% W_{ex} improved the TAN, un-ionized ammonia, nitrite and nitrate levels by 57.22%, 57.24%, 44.68% and 53.39%, respectively, relative to the values in ponds with the 20% W_{ex} .

The enhancement of the physicochemical parameters of water indicated that it was better to use a 30% W_{ex} than a 20% W_{ex} , as it provided more oxygen to the water and reduced the harmful residuals that have negative effects on water quality. In addition, the water physicochemical parameters vitally affected European sea bass performance. Many studies have reported that water quality (Setiadi *et al.*, 2018) such as a higher W_{ex} enhances water quality and reduces the concentration of harmful ammonia in culture ponds (Ng *et al.*, 1992; El-Saidy and Hussein, 2015). Furthermore, (Diana and Fast, 1989) found that a low SD represented the best water quality when it reduced ammonia and enhanced the DO concentration in ponds (Kpundeh, 2013).

It was obvious that both W_{ex} and SD significantly affected the growth performance of European sea bass, and the 30% W_{ex} treatments produced an improved WG of 15% and improved SGR of 12.5%. Additionally, the yield values were approximately 30% better at 30% W_{ex} than at 20% W_{ex} . These results showed that the 30% W_{ex} enhanced the growth performance of fish. Additionally, the LD group had better growth because it provided more space for fish and produced higher water quality. The LD treatment significantly improved the growth of tested European sea bass, by improving WG by 31% compared with that in the HD group.

Moreover, the interaction between SD and W_{ex} significantly affected growth performance. The 30% W_{ex} reduced the negative effect of HD relative to the effects with 20% W_{ex} at the same density. These results indicate that SD was the major factor affecting the growth performance and water quality was improved by increasing the W_{ex} . This conclusion was supported by the current results of European sea bass grown

Table (6). The effect of different stocking densities and two water exchange rates on serum and plasma parameters of European sea bass fingerlings.

Variable	20% water exchange rate			30% water exchange rate			Col1	P-value groups	C×G ¹
	High density	Mid density	Low density	High density	Mid density	Low density			
RBCs (10 ⁶ mm ⁻³)	1.06 ± 0.05 ^h	1.23 ± 0.01 ^g	1.28 ± 0.045 ^g	1.45 ± 0.05 ^f	1.67 ± 0.05 ^d	1.84 ± 0.03 ^c	<0.001	<0.001	0.005
Hb g/dL ³	5.9 ± 0.09 ^g	6.12 ± 0.01 ^{fg}	6.37 ± 0.04 ^f	7.48 ± 0.035 ^d	8.36 ± 0.15 ^c	9.01 ± 0.10 ^b	<0.001	<0.001	<0.001
Hct(%)	15.5 ± 0.17 ^f	16 ± 0.11 ^{ef}	16.3 ± 0.035 ^{ef}	20.4 ± 0.7 ^d	23.4 ± 0.02 ^c	24.4 ± 0.25 ^c	<0.001	<0.001	0.02
WBCs (10 ⁶ mm ⁻³)	20.5 ± 0.41 ^h	20.4 ± 0.01 ^h	21.3 ± 0.065 ^{gh}	26.2 ± 0.04 ^e	26.2 ± 0.00 ^e	30 ± 0.12 ^d	<0.001	<0.001	<0.001
Lymph (%)	45.8 ± 0.17 ⁱ	46 ± 0.1 ⁱ	46.8 ± 0.11 ⁱ	64.1 ± 0.12 ^e	66.8 ± 0.34 ^d	70.8 ± 0.50 ^b	<0.001	<0.001	0.001
Monocyte (%)	1.92 ± 0.05 ⁱ	2.1 ± 0.01 ^{gh}	2.11 ± 0 ^{gh}	2.22 ± 0.01 ^{fg}	2.38 ± 0.05 ^{de}	2.54 ± 0.03 ^{bc}	<0.001	<0.001	0.657
Ntrph (%)	15.6 ± 0.22 ^h	16 ± 0.04 ^g	16.1 ± 0 ^g	19.2 ± 0 ^d	20.3 ± 0.05 ^c	21.3 ± 0.06 ^b	<0.001	<0.001	<0.001

Values are means ± SEM, n = 3 per treatment group.

Means in a row without a common superscript letter differ (P < 0.05) as analyzed by two-way ANOVA and the TUKEY test.

1W × G = W ex L × group interaction effect.

in the HD group with the 20% W_{ex} , which had the lowest growth performance parameter values. On the other hand, the yield in the HD ponds with the 30% W_{ex} had the best value and was 35% better than the yield in HD ponds with the 20% W_{ex} .

Furthermore, the growth performance values were positively correlated with the feed utilization indicators. European sea bass grown in ponds with a 30% W_{ex} had a slightly better FCR, and the value decreased by 8% in the 20% W_{ex} treatment. This enhancement may be resulted in optimum water temperature which is supplied by this treatment. This improvement was more obvious in the PER and PPV values. Increasing the W_{ex} positively affected the protein utilization of juvenile European sea bass. In the same context, LD led to better feed utilization. Once more, the effect was clear for PPV, which was 60% higher in the LD group than in the HD group. Although the interaction between the two tested factors was not related to any significant differences, the 30% W_{ex} in the MD group produced the best FCR, FI, PER and PPV results. These results indicate that appropriate water quality, which was supported by the 30% W_{ex} , significantly affected the feed utilization of juvenile European sea bass. According to **Ercan *et al.* (2015)** and a review by **Kousoulaki *et al.* (2015)**, the FCR was highly related to environmental conditions, especially water temperature, a water temperature of 19.3°C produced a FCR of 1.05, and a water temperature of 14.1°C produced FCR values of 2.65 and 3.73 under tank conditions and a feeding regime of 3 meals/day to satiation (**Azzaydi *et al.*, 2000; Güroy *et al.*, 2013**). Correspondingly, (**Vinagre *et al.*, 2009; Lanari *et al.*, 2016**), who studied European sea bass, and (**Remen *et al.*, 2015**), who studied sea bream, reported that feed efficiency increased linearly with increasing water temperature up to 5°C when the preference range of juvenile European sea bass was 20-25°C (**Lupatsch *et al.*, 2001; Lupatsch *et al.*, 2003**). The 30% W_{ex} treatment was accompanied by higher water temperatures regardless of SD; thus, growth performance and feed utilization were improved. The same results were obtained for *Oreochromis niloticus* by **El-Saidy and Hussein (2015)**, who investigated the effects of both SD and W_{ex} on fish performance. They stated that a LD (50 fish/m³) produced the best values of growth performance and feed utilization parameters. However, they reported that the W_{ex} and the interaction between the SD and W_{ex} did not significantly affect fish growth performance or feed utilization. These results are in agreement with those of **Leal *et al.* (2011)**, who stated that a HD negatively affected the feed conversion efficiency (FCE) of juvenile European sea bass. **Roque d'Orbcastel *et al.* (2009)** revealed that a low FI led to a low SGR when their results showed that high SDs (70 and 100 kg/m³) led to the lowest FI and SGR values. Furthermore, these findings were highly related to water quality when the lowest specific flow rate occurred at the highest density (100 kg/m³). These findings are in agreement with the current results, in that the highest SD led to the lowest FI and SGR values. Additionally, the authors reported that a HD achieved the best net and total production. Another study by **Yousif (2002)** reported that the SD and W_{ex} together significantly affected water quality and fish performance; specifically, the lowest density and the highest W_{ex} significantly enhanced water quality and, in turn, produced the best growth performance of *Oreochromis niloticus*.

In the same context, different treatments significantly affected the fish body contents of protein, lipids and ash. The best protein content was recorded in European sea bass grown in the LD group with the 30% W_{ex} . These results are well-matched with the results of PPV, which was the highest in the same treatment. Nevertheless, lipids and ash tended to be the highest in the 20% W_{ex} treatment, especially under HD,

indicating that European sea bass grown in 20% W_{ex} ponds were exposed to stress, which increased the contents of lipids and ash (**El-Saidy and Hussein, 2015**).

The present data revealed that the different treatments had significant effects on haematological parameters, such as RBCs, Hb, Hct, WBCs, lymphocytes, monocytes, and neutrophils. These parameters are considered to provide vital evidence of stress, especially stress that results from environmental conditions (**Vosyliene, 1996; Graham, 1997; Montero *et al.*, 1999; Jawad *et al.*, 2004**) and infection (**Zorriehzahra *et al.*, 2010**).

A higher W_{ex} produced higher values for the haematological parameters. For example, the values obtained from the 30% W_{ex} treatment (38.66%, 35.07%, 42.75%, 32.42%, 45.54%, 16.10% and 27.60%, respectively) were greater than those obtained from the 20% W_{ex} treatment. The higher W_{ex} maintained more suitable environmental conditions, such as temperature, which is considered a vital parameter that influences the biological and physiological performance of European sea bass. Additionally, the 30% W_{ex} treatment reduced the values of harmful nitrogen compounds, which in turn improved the haematological parameters. Furthermore, the LD treatment also had the best haematological parameter values (**Kpundeh, 2013**). These effects, together with water quality improvements, led to a decrease in the accumulation of feed residuals which in turn reduced the accumulation of ammonia compounds. Moreover, no significant differences were observed in the Hct between LD and MD groups, and no significant differences were found in the WBCs between MD and LD groups. However, these differences became significant when the effect of W_{ex} and SD were combined. These findings indicate that both SD and W_{ex} influence fish performance. The parameters were significantly highest in ponds with a 30% W_{ex} and LD. Additionally, there were significant differences between all interaction conditions (SD and W_{ex}) when comparing the values of RBCs, Hb, Hct, monocyte and neutrophils. Some authors have shown that haematological parameters are directly affected by environmental conditions, such as temperature and oxygen availability, which directly affect haemoglobin and oxygen transport (**di Prisco and Tamburrini, 1992; Orun *et al.*, 2003; Arnaudova *et al.*, 2008; Tavares-Dias *et al.*, 2008; Sutthi *et al.*, 2018**). **Jawad *et al.* (2004)** revealed that the Hb and RBC values were highly correlated with the growth parameters and weights of fish. In this study, the highest FBW was recorded in fish grown in the LD group with the 30% W_{ex} , and the haematological parameter values tended to be highest in this treatment. Moreover, **Svobodova *et al.* (2008)** reported that more active fish tended to have higher values of haematological parameters, such as RBCs and Hb. The present study revealed that high water quality, which was provided by the 30% W_{ex} and LD treatment, achieved the best haematological parameters and produced the best environmental conditions in ponds. Additionally, the WBC results indicated that the highest value was recorded in European sea bass grown in the LD group with the 30% W_{ex} . **Weiss and Wardrop (2011)** stated that fish with higher WBC levels can tolerate infection better than fish with lower WBC levels. Likewise, (**Berillis *et al.*, 2016**) found that improving water quality by aeration led to a slight increase in monocytes and a decrease in lymphocytes but without any significant differences. In addition, (**Adeyemo, 2007**)

stated that neutrophils decreased significantly when fish were exposed to an environmental stressor such as lead.

Conclusion:

A higher W_{ex} maintains better water quality and reduces the negative effect of high SD. This improvement in water quality had a significant positive influence on European sea bass performance. The raise of fish production starts mainly with controlling the physicochemical water quality hence it is the main reason of successful sustainable aquaculture.

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